Figure 1: Nucleotide (SEQ ID NO: 1) and deduced amino acid sequence of human ChemR23 (AC075748)

1 175	_	E GAG	D GAT	E GAA		Y TAC					S AGT			D GAT	E GAA	15 219
16 220		P CCT		Y TAT		D GAC	S TCC		V GTG		L TTG	E GAG	D GAC	L TTA	S TCC	30 264
		L TTG				V GTG						V GTG	V GTG		Y TAC	45 309
46 310		I ATC	V GTC	C TGC	F TTC	L CTC	G GGG	I ATT	L CTG	G GGC	N AAT	G GGT	L CTG	V GTG	I ATC	60 354
61 355	I ATC	I ATT	A GCC	T ACC		K AAG	M ATG			T ACA	V GTG	N AAC	M ATG	V GTC		75 399
76 400			N AAC	L CTG		V GTG					F TTC	N AAC	V GTC	F <sub>.</sub> TTC	L CTC	90 444
91 445	P CCA	I ATC	H CAT	I ATC	T ACC	Y TAT	A GCC	A GCC	M ATG	D GAC	Y TAC	H CAC	W TGG	V GTT	F TTC	105 489
	G GGG		A GCC			K AAG				F TTC		L CTC	I ATC	H CAC	N AAC	120 534
121 535		F TTC	T ACC	S AGC	V GTC	F TTC	L CTG	L CTG	T ACC	I ATC	I ATC	S AGC	S TCT	D GAC	R CGC	135 579
136 580		I ATC				L CTC				S TCC	Q CAG	N AAC	H CAC	R CGC	s AGC	150 624
151 625		R CGC	L CTG	A GCT	Y TAC	M ATG		C TGC	M ATG	V GTC	I ATC	W TGG	V GTC	L CTG	A GCT	165 669
166 670	F TTC	F TTC	L TTG	S AGT	S TCC	P CCA		L CTC	V GTC	F TTC	R CGG	D GAC	T ACA	A GCC	N AAC	180 714
181 715	L CTG					S TCC				n AAC	F TTC	S AGC	L CTG	S TCC	T ACA	195 759
						W TGG				-	Q CAA			_	V GTG	210 804
			s AGC			M ATG							F TTC			225 849
						V GTC							Y TAC		T ACC	240 894
						Q CAG						K AAG		K AAG	K AAG	
						V GTG						F TTC			C TGC	270 984

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271	W	C	P	Y	H	T	L	N	L	L	E	L	H	H	T	285
985	TGG	TGC	CCC	TAC	CAC	ACA	CTC	AAC	CTC	CTA	GAG	CTC	CAC	CAC	ACT	1029
286	Α	M	P	G	s	V	F	s	L	G	L	P	L	Α	T	300
1030																
301	A	L	A	I	Α	N	s	C	M	N	P	I	L	Y	V	315
1075	GCC	CTT	GCC	TTA	GCC	AAC	AGC	TGC	ATG	AAC	CCC	ATT	CTG	TAT	GTT	1119
316	F	M	G	Q	D	F	K	K	F	K	V	A	L	F	S	330
1120	TTC	ATG	GGT	CAG	GAC	TTC	AAG	AAG	TTC	AAG	GTG	GCC	CTC	TTC	TCT	1164
331	R	L	V	N	Α	L	S	E	D	T	G	H	S	S	Y	345
1165	CGC	CTG	GTC	AAT	GCT	CTA	AGT	GAA	GAT	ACA	GGC	CAC	TCT	TCC	TAC	1209
346	P	S	H	R	S	F	T	K	M	S	s	M	N	E	R	360
1210	CCC	AGC	CAT	AGA	AGC	TTT	ACC	AAG	ATG	TCA	TCA	ĀТG	TAA	GAG	AGG	1254
361	T	s	M	N	E	R	E	T	G	M	${f L}$	*		•		372
1255	ACT	TCT	ATG	AAT	GAG	AGG	GAG	ACC	GGC	ATG	CTT	TGA				1290

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Figure 2: Amino acid sequence of human ChemR23 (371 amino acids) (SEQ ID NO 2). The seven predicted transmembrane domaines are underlined. The consensus sequence for *N*-linked glycosylation (N-X-S/T) in the N terminus is bold and the potential site of phosphorylation by PKC (S/T-X-R/K) in the C terminus is in italic.

MEDEDYNTSISYGDEYPDYLDSIVVLEDLSPLEARVTRIFLVVVYSIVCFLGILGNGLVIIIAT

FKMKKTVNMVWFLNLAVADFLFNVFLPIHITYAAMDYHWVFGTAMCKISNFLLIHNMFTSVFLL

TIISSDRCISVLLPVWSQNHRSVRLAYMACMVIWVLAFFLSSPSLVFRDTANLHGKISCFNNFS
LSTPGSSSWPTHSQMDPVGYSRHMVVTVTRFLCGFLVPVLIITACYLTIVCKLQRNRLAKTKKP

FKIIVTIIITFFLCWCPYHTLNLLELHHTAMPGSVFSLGLPLATALAIANSCMNPILYVFMGQD

FKKFKVALFSRLVNALSEDTGHSSYPSHRSFTKMSSMNERTSMNERETGML

Figure 3: Nucleotide and deduced amino acid sequence of mouse dez (AC u79525 - SEQ ID NOs:3 and 4, respectively)

1 265	M ATG	E GAG	Y TAC	D GAC	A GCT	Y TAC	n aac	D GAC	S TCC	G GGC	I ATC	Y TAT	D GAT	D GAT	E GAG	15 309
16 310	Y TAC	S TCT	D GAT	G GGC	F TTT	G GGC	Y TAC	F TTT	V GTG	D GAC	L TTG	E GAG	E GAG	A GCG	S AGT	30 354
31 355	P CCG	W TGG	E GAG	A GCC	K AAG	V GTG	A GCC	P CCG	V GTC	F TTC	L CTG	V GTG	V GTG	I ATC	Y TAC	45 399
46 400	3 AGC	L TTG	.V GTG	C TGC	F TTC	L CTC	G GGT	L CTC	L CTA	G GGC	n AAC	G GGC	L CTG	V GTG	I TTA	60 444
61 445	V GTC	I ATC	A GCC	T ACC	F TTC	K AAG	M ATG	K AAG	K AAG	T ACC	V GTG	n Aac	T ACT	V GTG	W TGG	75 489
76 490	F TTT	V GTC	n aac	L CTG	A GCT	V GTG	A GCC	D GAC	F TTC	L CTG	F TTC	n aac	I; ATC	F TTT	L TTG	90 534
91 535	P CCG	M ATG	H CAC	I ATC	T ACC	Y TAC	A GCG	A GCC	M ATG	D GAC	Y TAC	H CAC	W TGG	V GTG	F TTC	105 579
106 580	G GGG	K AAG	A GCC	M ATG	C TGC	K AAG	I ATC	S AGC	N AAC	F TTC	L TTG	L CTC	S AGC	H CAC	n aac	120 624
121 625	M ATG	Y TAC	T ACC	S AGC	V GTC	F TTC	L CTG	L CTG	T ACT	V GTC	I ATC	S AGC	F TTT	D GAC	R CGC	135 669
136 670	C TGC	I ATC	S TCC	V GTG	L CTG	L CTC	P CCC	V GTC	W TGG	S TCC	Q CAG	n aac	H CAC	R CGC	S AGC	150 714
151 715	I	R	L	A	Y	M	T	C	S	A	V	W	V	L	A	165
	ATC	CGC	CTG	GCC	TAC	ATG	ACC	TGC	TCG	GCC	GTC	TGG	GTC	CIG	GCI	759
166	F TTC	F	L	s	S	P	s	L	v	F	R	D	T	A	N	180 804
166 760 181	F	F TTC H	L TTG G	S AGC K	S TCC	P CCG T	s TCC C	L CTT F	V GTC N	F TTC N	R CGG F	D GAC S	T ACC L	A GCC A	N AAC A	180
166 760 181 805	F TTC	F TTC H CAT	L TTG G GGG	S AGC K AAG	S TCC I ATA P	P CCG T ACC	S TCC C TGC	L CTT F TTC	V GTC N AAC H	F TTC N AAC	R CGG F TTC	D GAC S AGC V	T ACC L TTG V	A GCC A GCC	N AAC A GCG T	180 804 195
166 760 181 805 196 850	F TTC I ATT	F TTC H CAT E GAG	L TTG G GGG -S TCC	S AGC K AAG	S TCC I ATA P CCA	P CCG T ACC H CAT	S TCC C TGC P CCC	L CTT F TTC A GCC	V GTC N AAC H CAC	F TTC N AAC S TCG	R CGG F TTC Q CAA	D GAC S AGC V GTA	T ACC L TTG V GTT	A GCC A GCC S TCC	N AAC A GCG T ACA	180 804 195 849
166 760 181 805 196 850 211 895	F TTC I ATT P CCT G	F TTC H CAT E GAG Y TAC	L TTG G GGG -S TCC S AGC	S AGC K AAG S TCC R AGA	S TCC I ATA P CCA H CAC	P CCG T ACC H CAT V GTG	S TCC C TGC P CCC A GCG	L CTT F TTC A GCC V GTC	V GTC N AAC H CAC T ACT	F TTC N AAC S TCG V GTC	R CGG F TTC Q CAA T ACC	D GAC S AGC V GTA R CGC	T ACC L TTG V GTT F TTC	A GCC A GCC S TCC L CTT	N AAC A GCG T ACA C TGC	180 804 195 849 210 894
166 760 181 805 196 850 211 895 226 940	F TTC I ATT P CCT G GGG	F TTC  H CAT  E GAG  Y TAC  F TTC	L TTG GGGG -S TCC S AGC L CTG	S AGC K AAG S TCC R AGA I ATC	S TCC I ATA P CCA H CAC	P CCG T ACC H CAT V GTG V	S TCC C TGC P CCC A GCG F TTC	L CTT  F TTC  A GCC  V GTC  I ATC	V GTC N AAC H CAC T ACT	F TTC N AAC S TCG V GTC	R CGG F TTC Q CAA T ACC A GCC	D GAC S AGC V GTA R CGC C TGC	T ACC L TTG V GTT F TTC Y TAC	A GCC A GCC S TCC L CTT L CTT	N AAC A GCG T ACA C TGC	180 804 195 849 210 894 225 939 240 984
166 760 181 805 196 850 211 895 226 940 241 985	F TTC I ATT P CCT G GGG GGC I ATC	F TTC  H CAT  E GAG  Y TAC  F TTC  V GTC	L TTG G GGG S TCC S AGC L CTG F TTC K	S AGC K AAG S TCC R AGA I ATC K AAG	S TCC I ATA P CCA H CAC CTC CTG	P CCG T ACC H CAT V GTG V GTC	S TCC C TGC P CCC A GCG F TTC R CGC	L CTT F TTC A GCC V GTC I ATC	V GTC N AAC H CAC T ACT I ATC	F TTC N AAC S TCG V GTC T ACG L CTG	R CGGG F TTCC Q CAA T ACCC A GCCC A GCCC	D GAC S AGC V GTA R CGC TGC K AAG	T ACC L TTG V GTT F TTC Y TAC N AAC	A GCC A GCC S TCC L CTT L CTT K AAG	N AAC A GCG T ACA C TGC T ACC	180 804 195 849 210 894 225 939 240 984
166 760 181 805 196 850 211 895 226 940 241 985	F TTC  I ATT  P CCT  G GGG  GGC  I ATC  P CCC	F TTC  H CAT  E GAG  Y TAC  F TTC  V GTC  F TTC  C	L TTG G GGGG -S TCC S AGC L CTG F TTC K AAG	S AGC  K AAG  S TCC  R AGA  I ATC  K AAG	S TCC I ATA P CCA H CAC CTG I ATC	P CCG T ACC H CAT V GTG V GTC Q CAG	S TCC C TGC P CCC A GCG F TTC R CGC T ACC	L CTT F TTC A GCC V GTC I ATC N AAC	V GTC N AAC H CAC T ACT I ATC	F TTC N AAC S TCG V GTC T ACG L CTG I ATC	R CGG F TTC Q CAA T ACC A GCC T ACC	D GAC S AGC V GTA R CGC TGC K AAG	T ACC L TTG V GTT F TTC Y TAC N AAC F TTC	A GCC  A GCC  S TCC  L CTT  K AAG  L CTC	N AAC A GCG T ACA C TGC T ACC K AAG C TGC T T T T T T T T T T T T T	180 804 195 849 210 894 225 939 240 984 255 1029 270 1074

301	Α	v	A	I	Α	N	s	С	M	N	P	I	L	Y	V	315
1165	GCC	GTC	GCC	ATC	GCC	AAC	AGC	TGC	ATG	AAC	CCC	ATT	CTG	TAC	GTC	1209
316	F	M	G	H	D	F	R	K	F	K	v	A	L	F	s	330
1210	TTC	ATG	GGC	CAC	GAC	TTC	AGA	AAA	TTC	AAG	GTG	GCC	CTC	TTC	TCC	1254
331	R	L	A	N	Α	L	s	E	D	T	G	P	S	s	Y	345
1255	CGC	CTG	GCC	AAC	GCC	CTG	AGT	GAG	GAC	ACA	GGC	CCC	TCC	TCC	TAC	1299
346	P	s	H	R	S	F	T	K	M	s	s	L	N	E	K	360
1300	CCC	AGT	CAC	AGG	AGC	TTC	ACC	AAG	ATG	TCG	TCT	TTG	AAT	GAG	AAG	1344
361	A	s	v	N	E	K	E	T	s	T	L	*				372
1345	GCT	TCG	GTG	AAT	GAG	AAG	GAG	ACC	AGT	ACC	CTC	TGA				1380

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Figure 4: Nucleotide and deduced amino acid sequence of rat G-protein coupled chemoattractant-1 (AC NM\_022218 - SEQ ID Nos: 5 and 6, respectively).

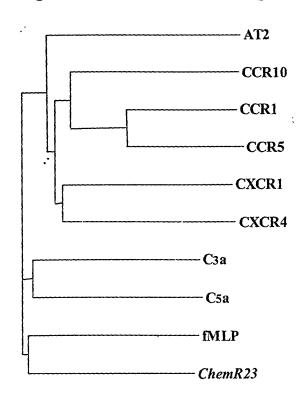
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	M ATG	E GAG	Y TAC								I ATC	Y TAC	G GGT	E GAG	E GAG		15 45
	Y TAT	s TCT	D GAC	G GGC	S TCG	D GAC		I ATC			L TTG	E GAG	E GAG	A GCG	G GGT		30 90
31 91	P CCA	L CTG	E GAG	A GCC	K AAG	V GTG		E GAG	V GTC	_	L CTG	V GTG	V GTA	I ATC	Y TAC		45 135
46 136		L TTG	V GTG	C TGC	F TTC		G GGG	I ATC	L CTA	G GGC	N AAT	G GGC	L CTG	V GTG	I ATT		60 180
	V GTC	I ATC	A GCC	T ACC	F TTC	K AAG		K AAG	K AAG	T ACG	V GTG	n aac	T ACC	V GTG	W TGG		75 225
76 226	-	V GTC		L CTG			A GCT					n aac	I ATC	F TTC	L TTG	٠.	90 270
	P CCC		H CAC	•			A GCC			D GAC	Y TAC	H CAC	W TGG	V GTG	F TTC		105 315
106 316	G GGG	K AAA	A GCC	M ATG			I ATT	S AGT	S AGC		L CTG	L CTA	S AĞC	H CAC	N AAC		120 360
	M ATG	Y TAC	T ACC	S AGC			L CTG		T ACT	V GTC	I ATC	S AGC	F TTC	D GAC	R CGC		135 405
136 406	C TGC	I ATC	S TCC	V GTG			CCC				Q CAG	N AAC	H CAC	R CGC	S AGC		150 450
	V GTG	R CGT	L CTG	A GCC	Y TAC	M ATG		C TGC			V GTC	W TGG	V GTC	W TGG	L CTT		165 495
166 496	S TCT	S TCT	E GAG	S TCT		P CCG	s TCC	L CTC	V GTC		G GGA	H CAC	V GTC	S AGC	T ACC		180 540
181 541	S AGC	H CAC	G GGG	K AAG	I ATA	T ACC	C TGC	F TTC	n aac	n Aac	F TTC	S AGC	L CTG	A GCG	A GCG		195 585
196 586	P CCC	E GAG	P CCT	F TTC	S TCT	H CAT	S TCC	T ACC	H CAC	P CCG	R CGA	T ACA	D GAC	P CCG	V GTA		210 630
211 631	G GGG	Y TAC	S AGC	R AGA	H CAT	V GTG		V GTC	T ACC	V GTC	T ACC	R CGC	F TTC	L CTC	C TGT		225 675
	G GGC										A GCC			L CTC			240 720
241 721	I ATC	V GTC	F TTC	K AAG	L TTG	_	R CGC	n aac		Q CAG	A GCC	K AAG	T ACC	K AAG	K AAG		255 765
		F TTC						I ATC			T ACC			L CTC	C TGC		270 810
271 811	W TGG	C TGC	P CCC	Y TAC		T ACA	L CTC		L CTG	L CTG	E GAG	L CTC	H CAC	H CAC	T ACG		285 855
286	A	v	P	A	s	v	F	s	L	G	L	P	L	A	T		300

856	GCT	GTG	CCA	GCC	TCT	GTC	TTC	AGC	CTG	GGA	CTG	CCC	CTG	GCC	ACA	900
301	Α	v	A	I	A	N	s	С	М	N	P	I	L	Y	V	315
901	GCC	GTC	GCC	ATC	GCC	AAC	AGC	TGT	ATG	AAC	CCC	ATC	CTG	TAC	GTC	945
316	F	М	G	H	D	F	K	ĸ	F	ĸ	v	A	L	F	s	330
946	TTC	ATG	GGC	CAC	GAC	TTC	AAA	AAA	TTC	AAG	GTG	GCC	CTT	TTC	TCC	990
331	R	L	V	N	A	L	s	E	D	T	G	P	s	s	Y	345
991	CGC	CTG	GTG	AAT	GCC	CTG	AGC	GAG	GAC	ACA	GGA	CCC	TCC	TCC	TAC	1035
346	P	s	н	R	s	F	T	ĸ	M	s	s	L	I	Ε	K	360
1036	CCC	AGT	CAC	AGG	AGC	TTC	ACC	AAG	ATG	TCC	TCA	TTG	ATT	GAG	AAG	1080
361	A	s	v	N	E	ĸ	E	т	s	Т	Ļ	*				372
1081	CCT	TCA	GTG	AAT	GAG	AAA	GAG	ACC	AGC	ACC	CTC	TGA				1116

## Figure 5: Alignment of ChemR23

Alignment of the amino acid sequence of ChemR23 with AT2 receptors, C3a, C5a and fMLP receptor and other chemoattractants related sequences were performed using ClustalX algorithm. Then, the dendrogram was constucted using TreeView algorithm.



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Figure 6: Nucleotide and deduced amino acid sequence of human Tig2 (AC Q99969 - SEQ ID Nos: 7 and 8, respectively)

1	M	R	R	L	L	I	P	L	A	L	W	L	G	Α	V	15
97	ATG	CGA	CGG	CTG	CTG	ATC	CCT	CTG	GCC	CTG	TGG	CTG	GGT	GCG	GTG	141
	G										~					30
142	GGC	GTG	GGC	GTC	GCC	GAG	CTC	ACG	GAA	GCC	CAG	CGC	CGG	GGC	CTG	186
	Q														W	45
															TGG	231
	A															60
	GCC														•	276
	P															75
														•	ACA	
	S		-													90
	AGC															366
	P															105
	CCC															411
	S														E	120
	TCT															456
	T															135
	ACC															501
	L														F	
															TTC	546
	P															164
547	CCT	GGA	CAG	TTC	GCC	TTC	TCC	AAG	GCC	CTG	CCC	CGC	AGC	TAA		588

Figure 7: Nucleotide and deduced amino acid sequence of mouse Tig2 (SEQ ID Nos: 9 and 10, respectively)

										V GTG	
								_		R AGG	30 191
 _	~		L CTG		_					V GTG	45 236
			E GAG							V GTG	60 281
			T ACC							Q CAG	75 326
~			K AAG				_	_	C TGC	T ACA	90 371
			R AGA							K AAA	105 416
			K AAA							P CCA	120 461
			P CCT	_							135 506
		_	G GGC					F TTC		P CCT	150 551
			s TCC								163 590

Figure 8: Amino acid sequence alignment of human and mouse TIG2. Identical and similar residues are shaded.

20 HUMAN : MERLIPLALULGAVGVG--VAELTEAQREGLQVALEEFHKHPPVCWAFQETSVE : 53
MOUSE : MECLISLALULGTVGTRGTEPELSETQRESLQVALEEFHKHPPVCLAFQEIGVD : 55 HUMAN : SAVDTPFPAGIFVRLEFKLQQTSCRKRDWKKPECKVRPNGRKRKCLACIKLGSED : 108
MOUSE : RAEEVLFSAGTFVRLEFKLQQTWCPKKDWKKPECTIKPNGRRRKCLACIKMDPKG : 110 120 HUMAN : KVLGRLVHCPIETOVLREAEEHQETQCLRVQRAGEDPHSFYFPGQFAFSKALPRS : 163 MOUSE: KILGRIVHCPILKO---GPCDPOELQCIKIAQAGEDPHGYFLPGQFAFSRALRTK: 162

151 168

mus HGYFLPGQFA FSRALRTK

rat RIYFFPGQFA FSRAL~~~

tig2 HSFYFPGQFA FSKALPRS

sus HSYYFPGQFA FFKALPPS

bos HSYYLPGQFA FIKAL~~~

gallus DVLYLPGMFA FSKGLP~~

## Identities :

W.						
		bos.pep	mus.pep	sus.pep	gallus	rat.pep
	tig2.pep	83.750	56.250	86.503	30.675	61.392
	bos.pep		54.375	87.500	31.875	56.329
	mus.pep			54.375	31.677	73.125
	sus.pep				31.288	58.228
	gallus.pep					30.818

Figure 11: Primary screening of HPLC fractions obtained from the fractionation of human ovary ascites.

The different fractions obtained following fractionation of human ovary ascites were diluted fivefold in the buffer assay and tested in aequorin assay using a cell line expressing ChemR23 (open circles) or cell lines expressing not related receptors (closed triangles and squares). The response obtained for each fraction was normalized using the ATP response of each cell line.

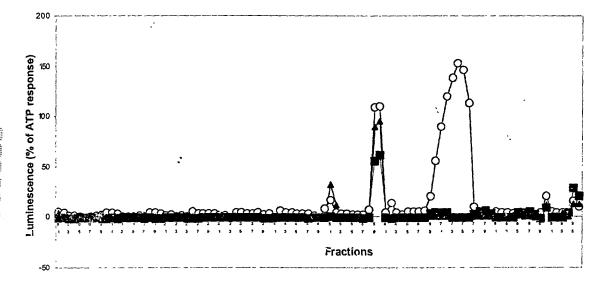
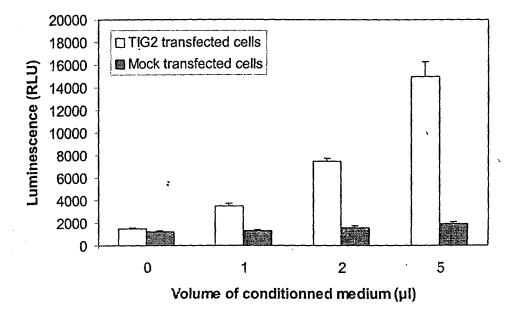


Figure 12: Activation of ChemR23 by cells transfected with TIG2

293 T cells were transiently transfected with pCDNA3- TIG2 or with pCDNA3 alone (mock transfected). Increasing volumes of the supernatant collected 4 days following transfection were analysed in a aequorin-based assay with CHO cells expressing ChemR23. A representative experiment is shown. Assay was performed in triplicate and SD are indicated.



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Figure 13: Characterization of antibodies directed against ChemR23

A mixture of recombinant cells made up of 2/3 recombinant ChemR23 CHO cells and 1/3 recombinant HCR CHO cells (negative control) was subject to react with either a supernatant of the anti ChemR23 5C 1H2 monoclonal antibody (thick line) or a supernatant with no known antibody activity (thin line, grey filling). After staining with FITC labeled anti mouse Ig these preparations were analysed by flow cytofluorometry. Results are displayed as a histogram of the number of cells (Events axis) expressing a given fluorescence (FL1-H axis). Monoclonal 5C 1H2 allowed to discriminate the ChemR23 recombinant sub-population of cells from the negative control cells as evidenced by the relative proportions of both type of cells. The background fluorescence of the assay is given by the second staining (grey filling).

